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Angiogenesis is essential for the growth and metastasis of solid tumors including breast cancer. In vitro and in vivo experimental models clearly demonstrate that suppressing angiogenesis leads tumor suppression. The overall goal of this proposal is to develop an adenovirus-based gene therapy approach for suppressing angiogenesis. In first year of the funding period, we have successfully constructed a human endothelial cell-targeted adenovirus gene delivery vector. In this second year of funding period, we successfully constructed endothelial cell-targeted adenoviral vector containing therapeutic genes including soluble VEGF receptor (sFlk and sFlt) and dominant negative angiogenesis-essential signaling molecules including Raf-1 and PI-3K. These vectors were found to significantly inhibit VEGF-induced human endothelial cell migration and in vitro angiogenesis. In the last year of the funding, we have focused on our effort to determine whether the endothelial cell-targeted adenoviral vector can be used to deliver anti-angiogenesis agents to block angiogenesis and tumor development in animal models. Our studies demonstrate that our approach can sufficiently inhibit both angiogenesis and breast tumor development. We believe that our endothelial cell target adenoviral vector provide a useful means for specifically delivering therapeutic agents to angiogenic tumors.

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Introduction

The survival, growth and metastasis of solid tumors including breast cancer depends on the formation of new blood vessels to provide tumors with nutrients and oxygen, a process called angiogenesis. In vitro and in vivo experimental models indicate that suppressing angiogenesis can also suppress solid tumors. However, the success of this approach largely depends on whether sufficient amounts of therapeutic agents can be delivered to tumor-associated endothelial cells without causing toxic effects to other tissues/cells. Our proposal is designed to develop an endothelial cell-targeted adenoviral vector and to use the targeted vector to express high levels of anticancer therapeutic genes in the sites of angiogenenic tumors specifically and efficiently.

Body

In the first year of this funding, we have developed an endothelial cell-targeted adenoviral vector (adenovirus fiber was modified by inserting NGR peptide in its HI-loop). In the second year of this funding, we constructed the endothelial cell-targeted adenoviral vector carrying anti-angiogenesis genes, soluble VEGF receptor (sFlk and sFlt) and dominant negative angiogenesis-essential signaling molecules [MEK1(-) and PI-3K(-)]. We further demonstrate that adenovirus-delivered sFlk and dominant negative PI-3K [PI-3K(-)] are capable of significantly blocking VEGF-induced cell migration and in vitro angiogenesis. In the final year of this funding, we determined the efficacy of endothelial cell-targeted adenovirus-delivered sFlk and dominant negative PI-3K to suppress breast tumor development in two established breast tumor models.

1. Athymic nude mouse model.

Exponentially growing MDA-MB-231 cells ($5X10^6$ cells/mouse) were subcutaneously injected at the inguinal mammary fat pad areas of 4-week old female athymic nude mice (Harlan Spague Dawley, Inc.) and tumors were closely observed. After four weeks (a period time to allow tumor formation), mice were divided into four groups (12 animals in each group): animals receiving control virus (containing no therapeutic gene) alone, animals receiving Ad/sFlk, animals receiving Ad/PI-3K(-), and animals receiving the combination of Ad/sFlk and Ad/PI-3K(-). The amount of Ad was given at 10^9pfu/animal. At four weeks after animals receiving Ad vectors, six animals from each group were sacrificed, then the tumors excised and weighed. Animals receiving Ad/sFlk, Ad/PI-3K(-) and combination of both Ad vectors displayed the reduction in tumor mass of 62%, 77%, and 89% respectively comparing to animals receiving the control virus (Fig.1). These results suggest that delivering anti-angiogenic genes to the angiogenic tumors is capable of suppressing breast cancer development.

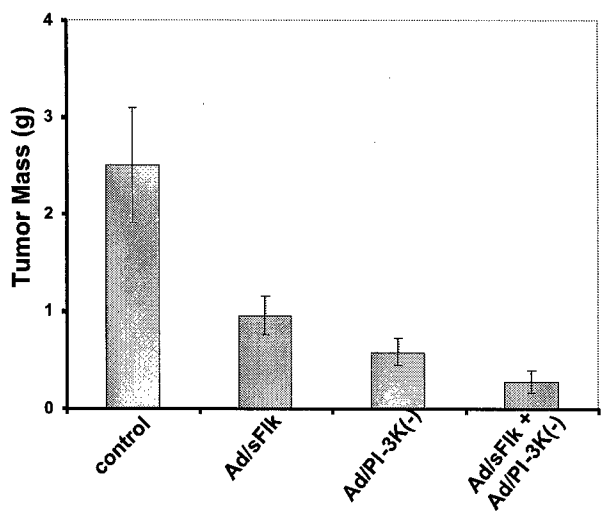

Fig.1. Endothelium -targeted Ad.sFlk and PI-3K(-) suppress breast tumor development in athymic nude mice. MDA-MB-231 cells were injected into animals for four weeks to allow tumor formation. Control Ad, Ad/ sFlk or Ad/PI-3K(-) were injected to the tumors and animals closely monitored for another 4 weeks. Animal s were sacrificed, tumors excised and weighed.

To determine the effect of endothelial cell-targeted adenovirus-delivered sFlk and PI-3K(-) on the survival of tumor-bearing nude mice, we monitored the remaining six

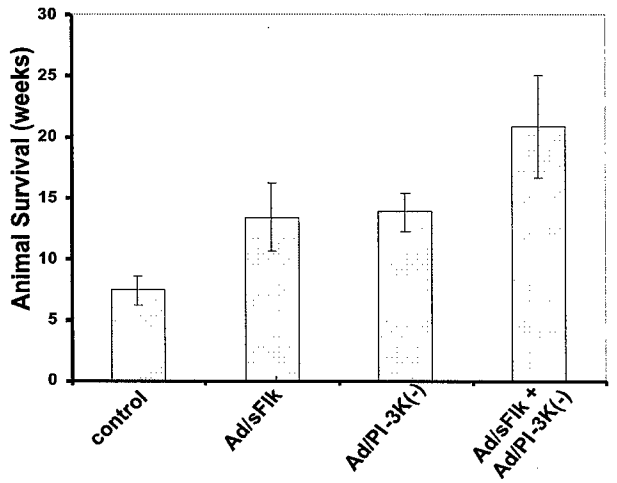

Fig.2. Endothelium -targeted Ad.sFlk and PI-3K(-) prolong breast tumor -bearing mouse survivals. MDA-MB-231 cells were injected into animals for four weeks to allow tumor formation. Control Ad, Ad/ sFlk or Ad/PI-3K(-) were injected to the tumors and animals closely monitored until they died. Note. One mouse receiving combination of Ad/ sFlk and Ad/PI-3K(-) still alive after 30 weeks.

animals in each group daily until their death. Animals receiving control virus lived average of another 7.4 weeks. In contrast, animals receiving Ad/sFlk and Ad/PI-3K(-) lived average of 13.4 and 13.8 weeks respectively (Fig.2). In six animals receiving the combination of both Ad vectors, we have one animals are still alive after 30 weeks. The five deceased mice lived average of 20.8 weeks (Fig.2). These results suggest that suppressing angiogenesis with Ad-delivered sFlk and PI-3K(-) can significantly prolong breast cancer-bearing animal survival.

2. Human/nude mouse model.

Four-week old nude mice were anaesthetized and a 2-cm^2 section of skin was surgically removed on the back of animals. A pre-cut section of fresh human neonatal foreskin was sutured into the place. The grafts were bandaged securely for 4 weeks to allow appropriate healing. MDA-MB-231 cells (5X10^{6}cells/mouse) were injected intradermally and five days later, control Ad, Ad/sFlk or Ad/PI-3K(-) were administrated through the tail veins at 10^{9}pfu/animal. After six weeks, mice were sacrificed, their grafts removed and analyzed for the presence of tumors. Six mice received control Ad grew tumors at the average mass of 464mg (Fig.3). In contrast, mice received Ad.sFlk and Ad.PI-3K(-) had tumors at the average mass of 264 and 171 mg respectively

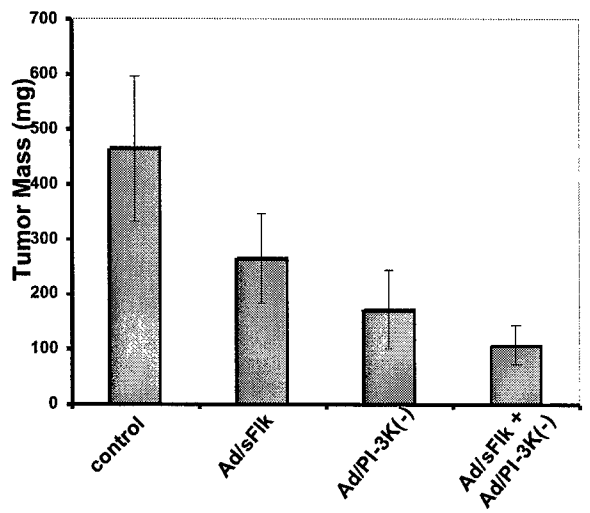

Fig.3. Endothelium -targeted Ad.sFlk and PI-3K(-) suppress breast tumor development on human skin graft. MDA-MB-231 cells were injected into the grafted human foreskin sutured on n ude mice for five days. Control Ad, Ad/ sFlk or Ad/PI-3K(-) were administrated to animals via tail veins. After 6 weeks, animals were sacrificed, grafts removed and analyzed for the presence of tumo rs. Note. Two mice receiving combination of Ad/ sFlk and Ad/PI-3K(-) did not grow tumors on the grafted skin.

(Fig.3). In mice receiving the combination of Ad.sFlk and Ad.PI-3K(-), we did not detect tumors in two of the six mice. The mice that did grow tumors had average mass of 107 mg. These results further demonstrate the effectiveness of endothelial cell-targeted Ad-delivered anti-angiogenic genes for suppressing breast tumors.

Key Research Accomplishment

We have demonstrated that the endothelial cell-targeted Ad vector containing sFlk and dominant negative PI-3K can effectively suppress breast tumor development and prolong the survival of tumor-bearing mice.

Reportable Outcomes

Two accepted and one submitted manuscripts were partially supported by this grant:

Yu, J., Bian, D., Mahanivong, C., Chang, R.K., Zhou, W., and **Huang, S.** (2004). p38 mitogen-activated protein kinase regulation of endothelial cell migration depends on urokinase plasminogen activator expression. *J.Biol.Chem.*, 279:50446-50454.

Li, H., Ye, X., Mahanivong, C., Bian, D., Chun, J., and **Huang, S.** (2005). Signaling mechanisms responsible for lysophosphatidic acid- induced urokinase plasminogen activator expression in ovarian cancer cells. *J.Biol.Chem.*, 280:10564-10571.

Mahanivong, C., Bian, D., Von Seggern D.J., and **Huang, S.** (2005). An efficient strategy to alter adenovirus tropism to facilitate human endothelial cell transduction. Submitted to *J.Virol.*

Conclusions

We have generated endothelial cell-targeted Ad vectors containing sFlk and dominant negative PI-3K. In our experiments, we found that these vectors can suppress VEGF-induced human endothelial cell migration and angiogenesis using in vitro experimental models. We further demonstrated that these Ad vectors were capable of efficiently suppressing breast tumor development in experimental animal models. These studies represent an alternative therapeutic modality for breast cancer treatment.